CD38+ Liver Stellate Cells in Chronic Hepatitis C Patients with Fibrosis

Titos Ahimsa, Rino Alvani Gani*, Ari Fahrial Syam**, Suhendro****

* Division of Hepatobiliary, Department of Internal Medicine
Faculty of Medicine, University of Indonesia/Dr. Cipto Mangunkusumo General National Hospital, Jakarta
** Division Gastroenterology, Department of Internal Medicine
Faculty of Medicine, University of Indonesia/Dr. Cipto Mangunkusumo General National Hospital, Jakarta
***Research and Development, Department of Internal Medicine
Faculty of Medicine, University of Indonesia/Dr. Cipto Mangunkusumo General National Hospital, Jakarta

Corresponding author:
Titos Ahimsa. Department of Internal Medicine, Cengkareng Hospital. Jl. Kamal Raya Bumi Cengkareng Indah Jakarta Indonesia. Phone/facsimile: +62-21-54372874. E-mail: dr.titosa @gmail.com

ABSTRACT

Background: *Approximately 3% of the world population is infected with hepatitis C virus (HCV). Protein of hepatitis C virus modulates apoptosis and steatosis, liver cell injury, activates liver stellate cells and liver fibrosis. Hepatitis C virus infection will cause injury to the hepatocytes. This injury to the hepatocyte will activate liver stellate cells. Stellate cells have a huge role in the development of liver fibrosis. The objective of this study is to evaluate the difference of active CD38+ liver stellate cells in various degree of fibrosis as well as its relation with aspartate transaminase (AST), alanine transaminase (ALT), and quantitative amount of hepatitis C virus ribonucleic acid (HCV RNA) in chronic hepatitis C.*

Method: *This study was a cross sectional study performed in 32 patients with chronic hepatitis C who had undergone liver USG, did not suffer from hepatoma, had undergone liver biopsy. Paraffin block of patients' liver tissue was further stained using haematoxylin and eosin technique to identify the metavir degree which is categorized into mild-moderate or severe degree. Special staining is performed to evaluate liver stellate cells that were then counted in averagely in five fields of view.*

Results: *In this study, we found significant difference in the amount of CD38+ stellate liver cells between severe and mild-moderate fibrosis ($p < 0.001$), there was no association between CD38+ stellate liver cells with AST ($p = 0.2$) or ALT ($p = 0.7$), and there was association between CD38+ stellate liver cells with quantitative HCV RNA ($r = -0.372$).*

Conclusion: *Total amount of CD38+ stellate liver cells in severe fibrosis was higher compared to the total amount of CD38+ liver stellate cells in mild-moderate fibrosis. There was no association between the value of AST, ALT, and quantitative HCV RNA with the number of CD38+ stellate liver cells.*

Keywords: *CD38+ stellate cells, chronic hepatitis C, aspartate aminotransferase (AST), alanine aminotransferase (ALT), viral load hepatitis C virus ribonucleic acid (HCV RNA)*

ABSTRAK

Latar belakang: *Sekitar 3% populasi di dunia terinfeksi virus hepatitis C. Protein virus hepatitis C memodulasi apoptosis dan steatosis, cedera sel hati, mengaktifkan sel stelata hati dan fibrosis hati. Infeksi virus hepatitis*

C akan menimbulkan cedera pada hepatosit. Cedera pada hepatosit ini akan mengaktivasi sel stelata hati. Sel stelata berperan besar pada proses perkembangan fibrosis hati. Tujuan penelitian ini adalah untuk mengetahui perbedaan jumlah sel stelata hati aktif CD38+ pada berbagai derajat fibrosis serta hubungannya dengan aspartate aminotransferase (AST), alanine aminotransferase (ALT), jumlah hepatitis c virus ribonucleic acid (HCV RNA) kuantitatif pada hepatitis C kronik.

Metode: *Penelitian ini merupakan studi potong lintang yang dilakukan pada 32 pasien hepatitis C kronik yang sudah dilakukan ultrasonografi (USG) hati dan tidak menderita hepatoma serta telah dilakukan biopsi hati. Paraffin block jaringan hati pasien selanjutnya diwarnai menggunakan teknik hematoksilin eosin untuk menilai derajat metavir yang dikategorikan menjadi derajat ringan-sedang atau berat. Pewarnaan khusus dilakukan untuk menilai sel stelata hati yang dihitung rata-rata pada lima lapangan pandang.*

Hasil: *Pada penelitian ini didapatkan perbedaan jumlah sel stelata hati CD38+ yang bermakna antara fibrosis derajat berat dan derajat ringan-sedang ($p < 0.001$), tidak didapatkan hubungan antara sel stelata hati CD38+ dengan AST ($p = 0,2$) maupun ALT ($p = 0,7$), dan tidak didapatkan hubungan antara sel stelata hati CD38+ dengan HCV RNA kuantitatif ($r = -0,372$).*

Simpulan: *Jumlah sel stelata hati CD38+ pada fibrosis berat lebih tinggi daripada jumlah sel stelata hati CD38+ pada fibrosis ringan-sedang. Tidak terdapat hubungan antara nilai AST, ALT dan HCV RNA kuantitatif dengan jumlah sel stelata hati CD38+.*

Kata kunci: *CD38+ sel stelata, hepatitis C kronik, aspartate aminotransferase (AST), alanine aminotransferase (ALT), viral load hepatitis c virus ribonucleic acid (HCV RNA)*

INTRODUCTION

Approximately 3% of the world population is infected with hepatitis C virus. Protein of the hepatitis C virus modulates apoptosis and steatosis, liver cell injury, activates liver stellate cells and liver fibrosis. Around 10-20% will develop into liver cirrhosis and hepatocellular carcinoma. While the body immune system tries to eliminate the virus, liver cells injury and fibrosis in chronic infection happen through direct cellular toxicity and release of long-term inflammatory cytokines.[1,2] Hepatitis C virus infection will cause injury to the hepatocytes. This injury to the hepatocytes will activate liver stellate cells. Activated stellate cells changes into cells such as myofibroblast which will then start the fibrogenesis process. Liver fibrosis is a reaction of granulation tissue development which is associated with injury to the liver parenchyma cells and occurs in the long-term. Fibrogenic response is marked by the accumulation of extracellular matrix that is high in collagen fibre and failure of matrix turn over process. Regression of fibrosis process can be achieved by ceasing the chronic injury process towards the liver cells. By ceasing the injury to liver cells, liver myofibroblast will disappear.[3] Although it has been known that other mesenchymal liver cells also contribute in the accumulation of extracellular matrix, stellate cells activation pathway is still considered as the main pathway in the process of liver fibrosis through its response towards liver tissue injury.[4] These cells have huge role in the development of liver fibrosis, thus the effort to the identify active stellate cells is an important thing in the process of understanding pathogenesis, therapy, and liver fibrosis evaluation.[5]

Stellate liver cell itself is a mesenchymal cell in the liver that has high degree of heterogenicity and plasticity; therefore it contains many compounds that can be used for identification, particularly by immunohistochemical staining. One of the potential immunohistochemical marker is CD38+, this is a cell membrane molecule of the active liver stellate cells which has been known to be expressed in rats' liver stellate cells in vitro and in vivo.[4-7] A study performed by Abdeen et al in chronic hepatitis patients revealed that CD38+ staining could be used to identify liver stellate cells and was a strong predictor for moderate and severe degree of fibrosis. A study by El-Gendi et al in chronic hepatitis C patients proved that CD38+ liver stellate cells count was strongly correlated with METAVIR activity and fibrosis score.[8]

In identifying METAVIR fibrosis score from mild to severe, CD38+ staining technique has better specificity compared to α-SMA, which is 95.45% vs. 77.27% with lower sensitivity, which was 90% vs. 95%. Additionally, the positive predictive value of CD38+ is 94.7%, while α-SMA is 79.2. The number of chronic hepatitis C (CHC) virus affects the injury in hepatocytes. This number of CHC virus is measured in the form of quantitative HCV RNA. Hepatocyte

cell which is injured will release transaminase enzyme in the form of aspartate aminotransferase (AST) and alanine aminotransferase glutamic (ALT) which can be measured in the blood.[4] According to Sandulescu et al, there was weak association between ALT and active liver stellate cells in chronic hepatitis C.[9,10] In the last few years, studies using CD38+ as a liver stellate cells marker was still rarely performed. This study aimed to identify the association between CD38+ liver stellate cells count and several clinical parameters in CHC patients, including degree of fibrosis, quantitative HCV RNA, AST and ALT values.

METHOD

This study was an observational analytic study and used cross sectional study design. Research would be performed in Cipto Mangunkusumo Hospital by analysing medical records. Target population in this study was chronic hepatitis C patients. Inclusion criteria in this study were chronic hepatitis C patients who had undergone biopsy, patient gave consent to participate in the study. Exclusion criteria of this study were presence of hepatocellular carcinoma in patients, hepatoprotector drugs consumption, viral infection which could increase AST-ALT, diabetes mellitus, fatty liver, not currently suffering from typhoid fever; not currently suffering from pulmonary tuberculosis. All samples included in this study would receive explanation before hand on the objectives, procedures to be performed, and complications that might happen in this study. Samples could only participate if they had given written consent. Variables that would be evaluated were number of CD38+ liver stellate cells, degree of fibrosis, AST value, ALT value, and quantitative HCV RNA.

Samples are study subjects who are part of the accessible population and have fulfilled the inclusion and exclusion criteria. Study samples were collected by identifying data of chronic hepatitis C patients who had undergone liver ultrasonography (USG), did not suffer from hepatoma, and had undergone liver biopsy. Further, paraffin block of these patients were searched in the Pathological Anatomy Department, Faculty of Medicine, University of Indonesia (FMUI)/ Cipto Mangunkusumo Hospital, and later the Metavir degree in these samples were identified. Results were categorized into mild or severe degree, and were further given specific staining for liver stellate cells. From the results of this staining, number of stellate liver cells would be counted in 5 fields of view and average would be counted.

Data collected from this study was number of liver stellate cells; metavir degree from paraffin block of liver biopsy, AST, ALT values; quantitative HCV RNA. Instrument being used in this study was paraffin block of the liver biopsy with the following steps: to all paraffin blocks taken for study subjects, unstained slide cutting would be performed for immunohistochemical staining with 4 micron thickness using avidin biotin methods; paraffin blocks which are ready were further evaluated for degree of inflammation and fibrosis; later for immunohistochemical staining of liver stellate cells, CD38+ was used. From these staining, we could count the amount of liver stellate cells per field of view (5 fields of view) later we counted the average amount and presented the results in numerical category.

Obtained research results would be analysed statistically to evaluate the correlation between independent and dependent variables. Data from the research results was further recorded and analysed using SPSS version 13. Data would be presented in the form of tables and figures.[3] Hypothesis testing was performed by bivariate analysis between independent variables and each dependent variables in numeric-categorical measurement scale for degree of fibrosis variables and numeric for AST, ALT, and quantitate HCV RNA variables. For data in numeric-numeric measurement scale, hypothesis testing being used was correlation test, particularly Pearson correlation test and Spearman correlation test. For data in numeric-categorical measurement scale, we performed analysis using Mann Whitney test. Ethical clearance for this study has been submitted and approved by Health Research Ethics Committee FMUI.

RESULTS

A total of 32 biopsy preparations of chronic hepatitis C patients, which consisted of 20 males and 12 females were included in this study. Patients aged more than 40 years old were 16 patients and those aged below 40 were 16 patients. Table 1 showed the distribution of subjects' characteristics based on sex and age.

Table 1. Distribution of subjects' characteristics based on sex and age (n=32)

Characteristics	n (%)
Sex	
Male	20 (62.5)
Female	12 (37.5)
Age	
< 40 years old	16 (50)
≥ 40 years old	16 (50)
Degree of fibrosis	
Mild-moderate	16 (50)
Severe	16 (50)

In this study, there was significant difference in the number of CD38+ stellate liver cells between severe and mild-moderate degree of fibrosis ($p < 0.001$) as shown in table 2.

Table 2. Difference in the number of CD38+ stellate liver cells based on the degree of fibrosis

Variable	Fibrosis		P value*
	Severe	Mild	
Number of CD38+ Stellate Liver Cells	5.3 (3.4-7.8)	1.2 (1.0-1.6)	< 0.001

*Mann Whitney Test

In this study, we reported no correlation found between CD38+ stellate liver cells with AST ($p = 0.2$) or even ALT ($p = 0.7$) value.

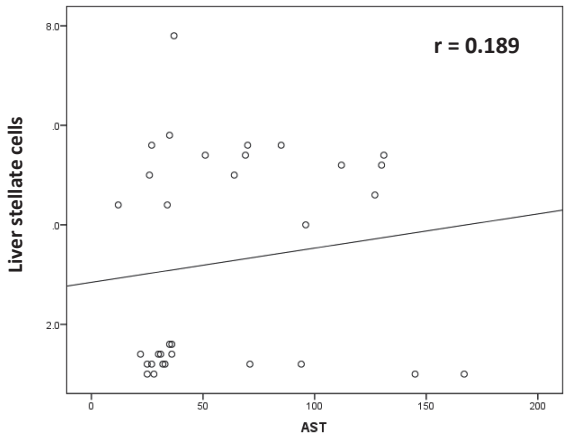

Figure 1. Correlation between CD38+ liver stellate cells and AST value

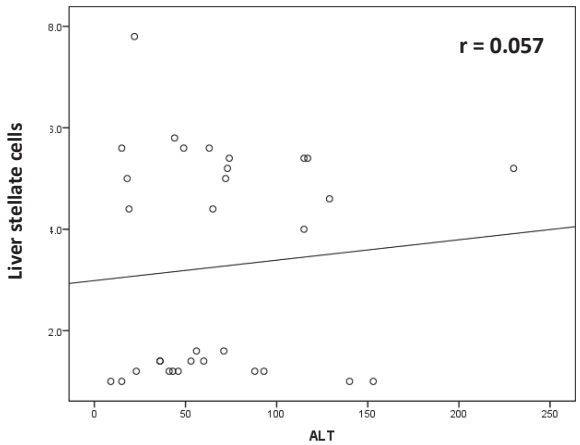

Figure 2. Correlation between CD38+ liver stellate cells and ALT value

Analysis results on the correlation of CD38+ liver stellate cells and quantitate HCV RNA reported that there was no correlation between CD38+ liver stellate cells and quantitative HCV RNA ($r = -0.372$).

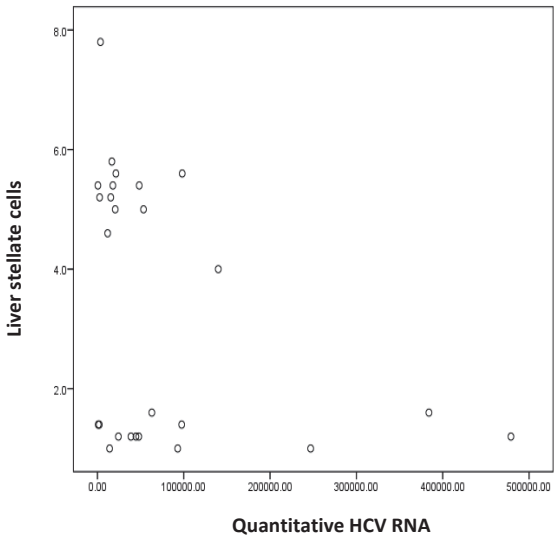

Figure 3. Correlation between CD38+ stellate liver cells and quantitative HCV RNA

DISCUSSION

In this study, we obtained higher number of CD38+ liver stellate cells in severe fibrosis compared to mild-moderate fibrosis. The average number of CD38+ liver stellate cells increased significantly ($p < 0.001$) in accordance with the severity degree of fibrosis from mild-moderate fibrosis with number of stellate cells of 1.2 (1.0–1.6) to severe fibrosis with number of stellate cells of 5.3 (3.4–7.8). Increase of CD38+ liver stellate cells in advancing degree of fibrosis was also in line with the results of previous study by El-Gendi et al.[7] The study aimed to confirm CD38+ examination as an immunohistochemistry marker ro detect stellate cells and evaluate the correlation between number of CD38+ and fibrosis score and semiquantitative assessment method towards activated stellate cells from 42 liver biopsy and using CD38+ and α-SMA immunostaning. Similar results were also found in the study performed by Abdeen et al.[8] The objectives of this study were similar to the study by El-Gendi et al. However, more samples were included compared to the study by El-Gendi et al. In the study conducted by Abdeen et al, 100 liver biopsies of chronic liver disease patients were studied. The results of this study also revealed the increase of CD38+ liver stellate cells in accordance with the advancing degree of fibrosis from mild to severe. This was consistent with the hypothesis that CD38+ liver stellate cells would increase in concordance with the advancing degree of fibrosis of the liver cells. A retrospective study, which was conducted by Tomanovic et al, studied 20 liver biopsy samples of chronic hepatitis C patients and 10 normal liver biopsy samples. Results showed that there

was positive correlation between degree of fibrosis with the number of active liver stellate cells.[1] In that study, it was concluded that activated liver stellate cells could be a prognostic marker for severe fibrosis and liver cirrhosis in chronic hepatitis C patients.

Results of correlation analysis between CD38+ liver stellate cells and AST in chronic hepatitis C patients in this study showed that there was no correlation between AST and number of CD38+ liver stellate cells in chronic hepatitis C patients ($p = 0.20$). Study conducted by Nadeem et al in 107 non-cirrhotic hepatitis C patients and had not received treatment for hepatitis C revealed that there was no association between AST value and degree of liver fibrosis which implicitly showed that there was no correlation between AST value and number of CD38+ liver stellate cells.[11] This could be caused by AST which is not only specific to represent condition of the liver but also that of the heart, skeletal muscle, kidney, brain, and red blood cells.[12] Meanwhile, for correlation analysis between CD38+ liver stellate cells and ALT in chronic hepatitis C patients that hepatocellular injury could lead to the release of cytosol alanine transaminase enzyme into the blood circulation. ALT value is often used as a marker of liver injury in chronic hepatitis C patients, which include assessment to progressivity, evaluation of therapy and prognosis.[13] In this study, the obtained results showed that there was no correlation between ALT and number of CD38+ liver stellate cells in chronic hepatitis C patients ($p = 0.70$). Study performed by Pradat et al to 864 positive HCV RNA patients with normal ALT value for 6 months which was measured from the time of liver tissue collection to ALT value before administration of therapy.[14] Normal ALT value was defined as value less than upper normal limit, while persistent normal ALT was defined as continuous normal value which was obtained for 6 months. The study revealed that there was no correlation between degree of liver fibrosis and ALT in chronic hepatitis C patients before receiving therapy. This could be caused by the activity of ALT which changes easily, fluctuative, and influenced by other factors, such as sex, body mass index. In chronic hepatitis C patients, liver cell death can occur through apoptosis and necrosis, thus the cells that are almost dead produce less ALT. This explains why there was weak correlation between increase of ALT and degree of liver fibrosis.[13] Puoti et al also studied 46 carrier hepatitis C patients with persistent normal ALT and compared them with 52 positive HCV RNA patients with increased ALT value.[15] Results of this study revealed that there was no difference between patients with normal ALT value and patients with increased ALT value, therefore it was concluded that there was no correlation between the degree of liver fibrosis and ALT values. Thus, by the absence of correlation between degree of liver fibrosis and ALT value, indirectly it showed that there was no correlation between CD38+ liver stellate cells and ALT value. Number of publications which opposed this assertion encouraged Kyrlagkitsis et al to conduct a retrospective study to 91 chronic hepatitis C patients with persistent normal ALT value and to 94 patients with abnormal liver biochemistry by comparing liver histology appearance from each groups.[16] Development of fibrosis was assessed as early as the time of infection. Researchers reported one from six chronic hepatitis C patients with persistent normal ALT value significantly experienced progressive liver disease. They also found lesser degree of fibrosis in patients with normal ALT compared to patients with abnormal ALT value.[13]

Assessment of correlation between quantitative HCV RNA and CD38+ liver stellate cells in chronic hepatitis C patients showed that there was no correlation between quantitative HCV RNA and CD38+ liver stellate cells. This was similar with the cohort study conducted by Fanning et al to 77 chronic hepatitis C patients by monitoring clinical parameter, biochemistry, and histology.[17] The results of the study revealed that there was no significant correlation between quantitative HCV RNA with degree of liver fibrosis.[17] Study performed by Romeo et al also reported similar result. The study was conducted to 170 chronic hepatitis C patients and 27 hepatocellular carcinoma patients who had undergone liver biopsy. The results of the study exhibited that advancement of hepatitis C disease was not associated with genotype difference or HCV RNA level.[18] Deterioration of hepatitis C disease was in accordance with the development of liver fibrosis, which was also in conjunction with the increase of liver stellate cells. Hence, we could conclude that HCV RNA level was not correlated with liver stellate cells.

CONCLUSION

From this study, we can conclude that the number of CD38+ stellate liver cells in severe fibrosis was higher compared to the number of CD38+ stellate liver cells in mild-moderate fibrosis. The correlation between AST and number of CD38+ stellate liver cells; between ALT and number of CD38+ stellate liver cells and between quantitate HCV RNA and number of CD38+

stellate liver cells were not found. Therefore, further studies are needed to develop soluble CD38+ from the blood serum as biomarker in liver fibrosis; role of active CD38+ stellate cells in chronic hepatitis C with normal ALT value and further studies are needed to identify the association between quantitative HCV RNA with other cells, to prevent the development of liver cirrhosis.

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